

## Construction of Pbs.PGK.PCRI

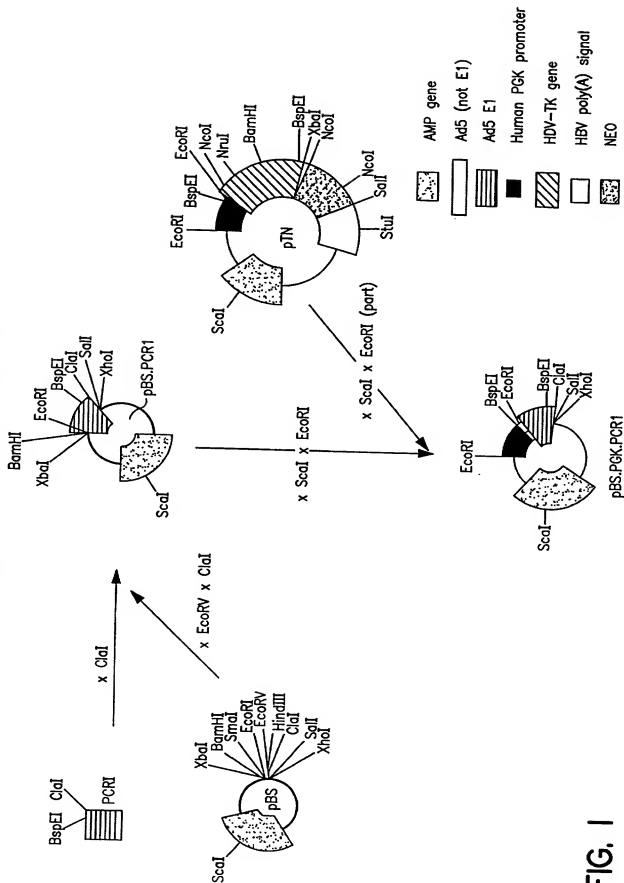


FIG. 1



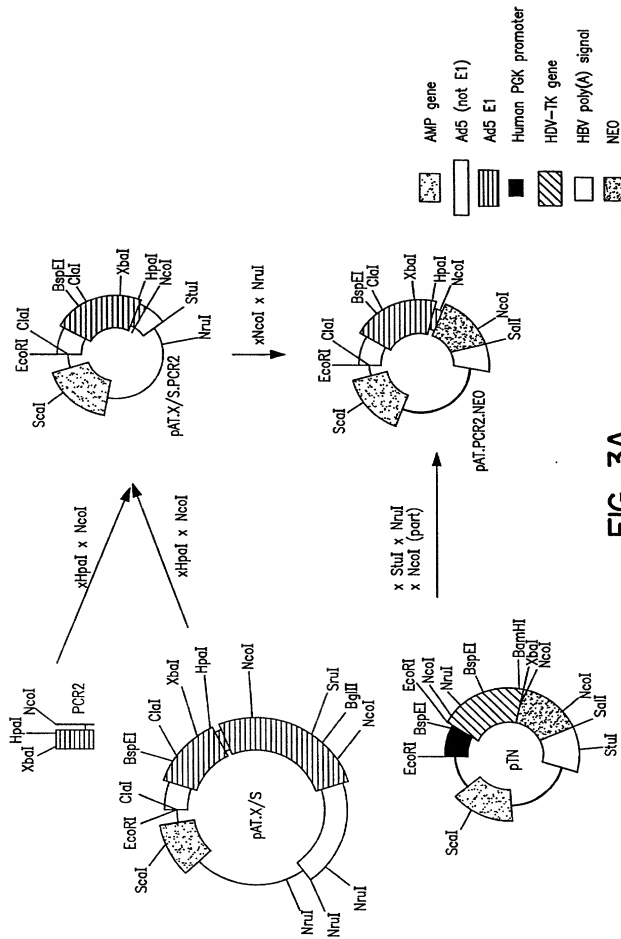


FIG. 3A

# Construction of pIG.E1a.NEO

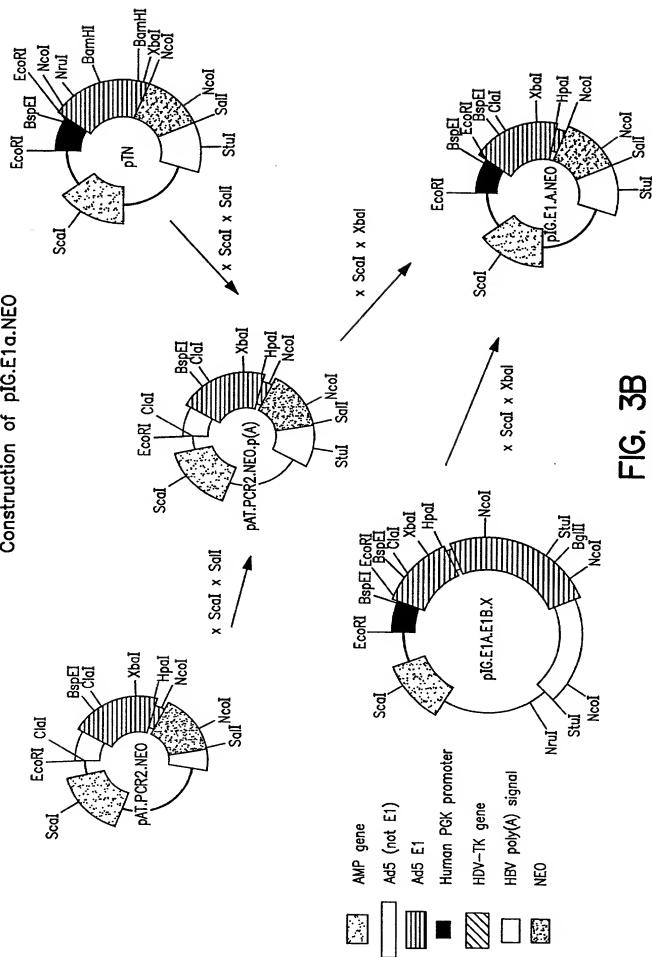
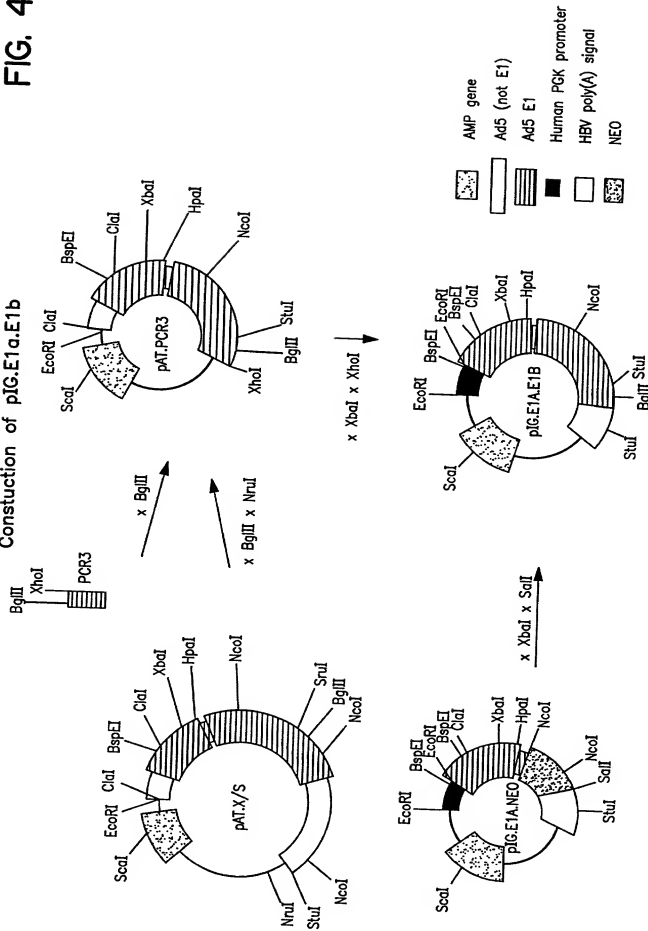


FIG. 3B

## Construction of pIG.E1a.E1b

FIG. 4



## Construction of pIG.NEO

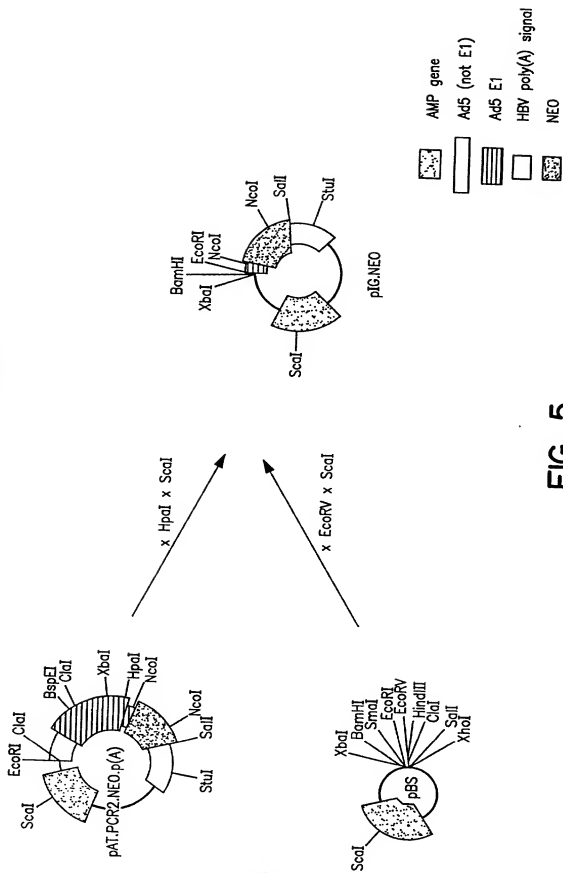
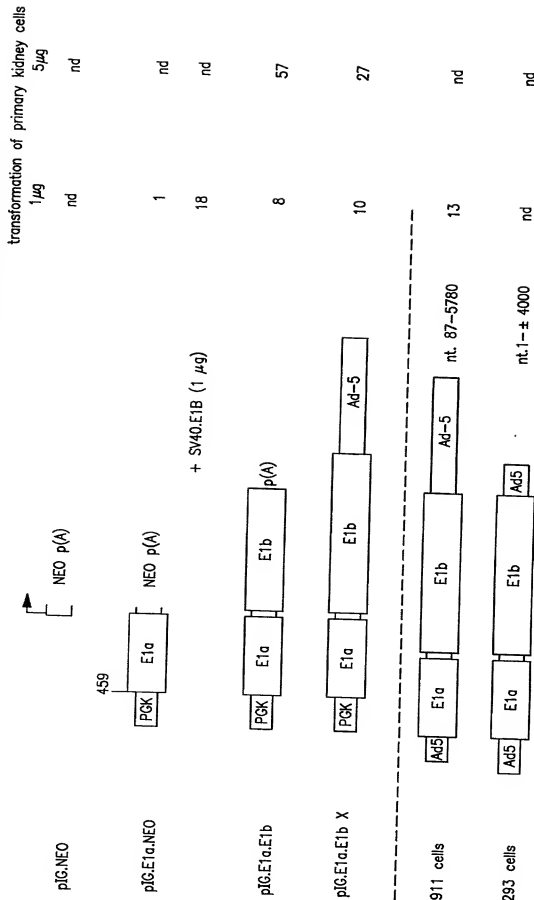


FIG. 5

# Overview of available adenovirus packaging constructs and assessment of their capacity to transform primary kidney cells



\*average of 5 plates 21 days after transfection

FIG. 6

Western blotting analysis of A549 clones transfected with pIG.E1A.NEO and PER clones (HER cells transfected with pIG.E1A.E1B)

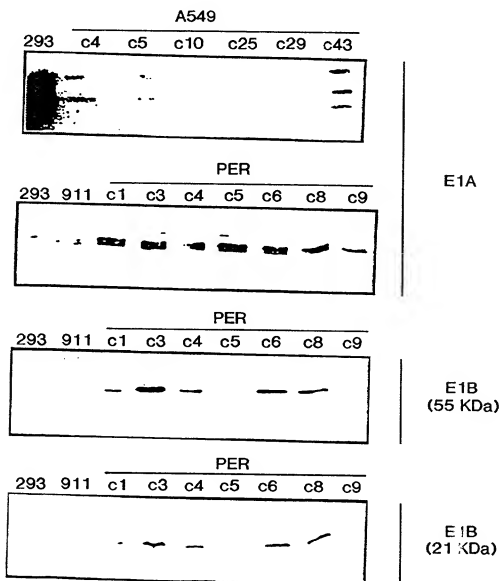


FIG. 7



# Southern blot analyses of 293, 911 and PER cell lines

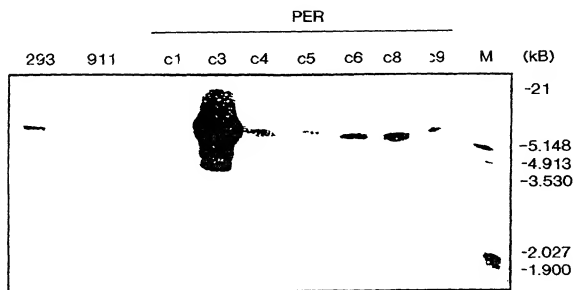


FIG. 8

Transfection efficiency of PER.C3, PER.C5, PER.C6 and 911 cells. Cells were cultured in 6-well plates and transfected (n=2) with 5  $\mu$ g pRSV.lacZ by calcium-phosphate co-precipitation. Forty-eight hours later the cells were stained with X-GAL. The mean percentage of blue cells is shown.

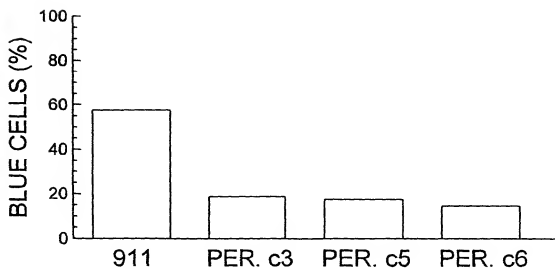


FIG. 9

# Construction of pMLP1.TK

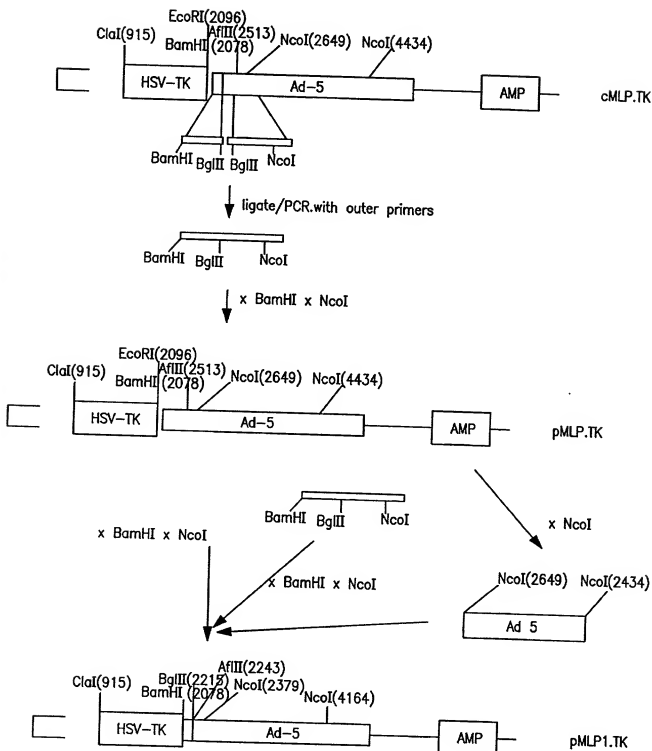
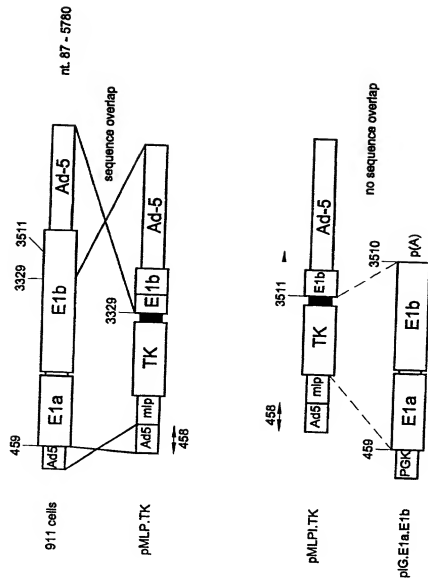


FIG. 10

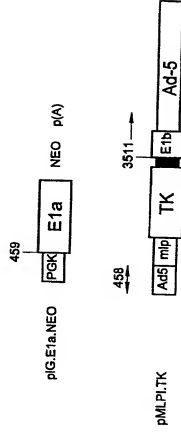
# New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on primary cells

FIG. 1IA

New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on established cell lines: transfection with E1a and selection with G418 **FIG. 1 B**

# Generation of recombinant adenovirus

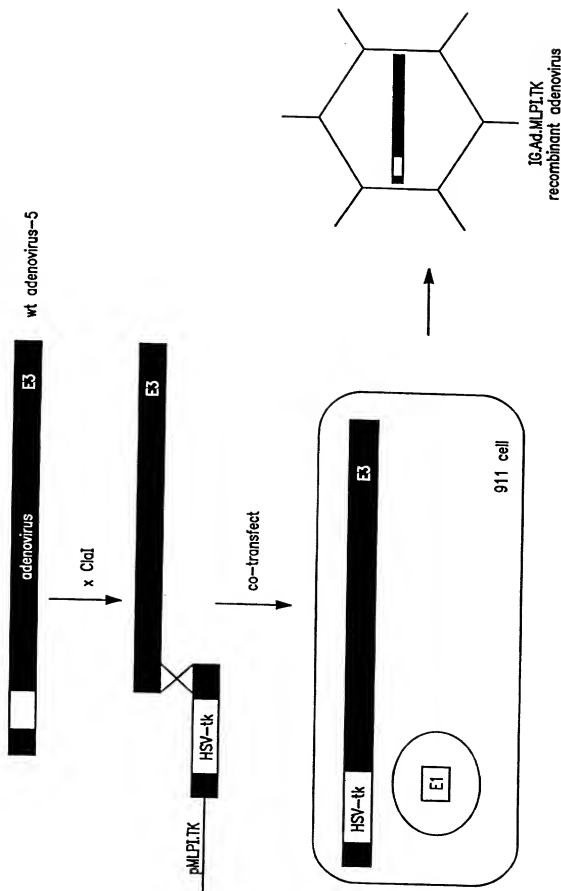


FIG. 12

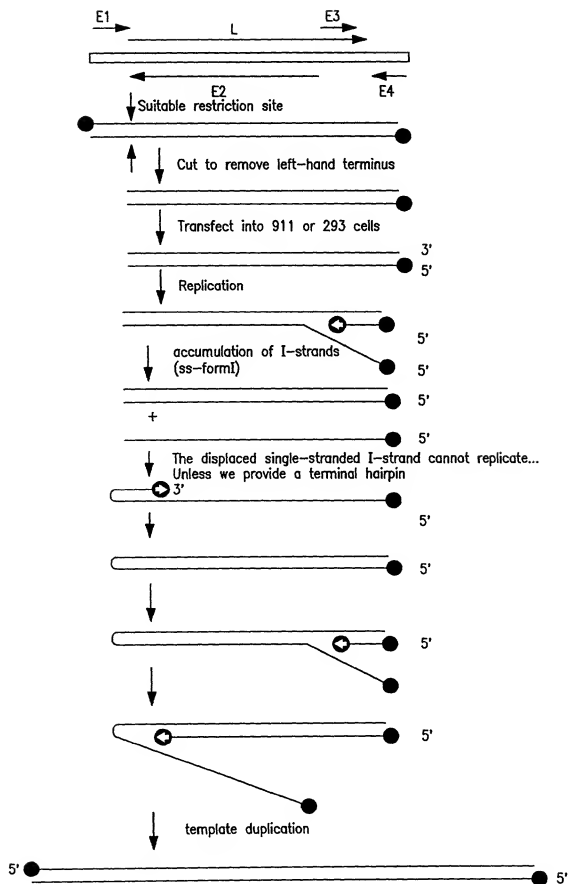


FIG. 13

# Replication of Adenovirus

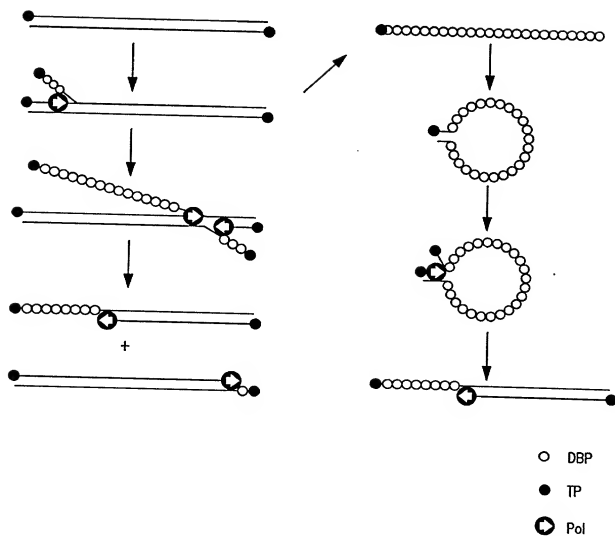


FIG. 14



The potential hairpin conformation of a single-stranded DNA molecule that contains the HP/asp sequences used in these studies. Restriction with the restriction endonucleases *Asp718I* of plasmid pICLHa, containing the annealed oligonucleotide pair HP/asp1 en HP/asp2 will yield a linear double-stranded DNA fragment. In cells in which the required adenovirus genes are present, replication can initiate at the terminus that contains the ITR sequence. During the chain elongation, the one of the strands will be displaced. The terminus of the single-stranded displaced-strand molecule can adopt the conformation depicted above. In this conformation the free 3'-terminus can serve as a primer for the cellular and/or adenovirus DNA polymerase, resulting in conversion of the displaced strand in a double-stranded form.

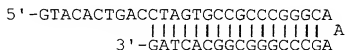


FIG. 15

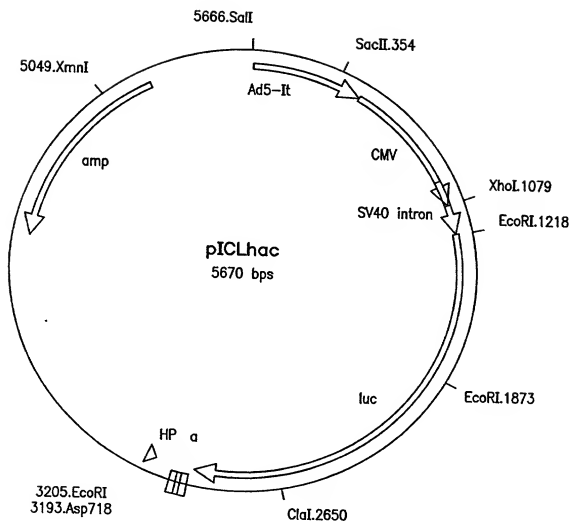


FIG. 16

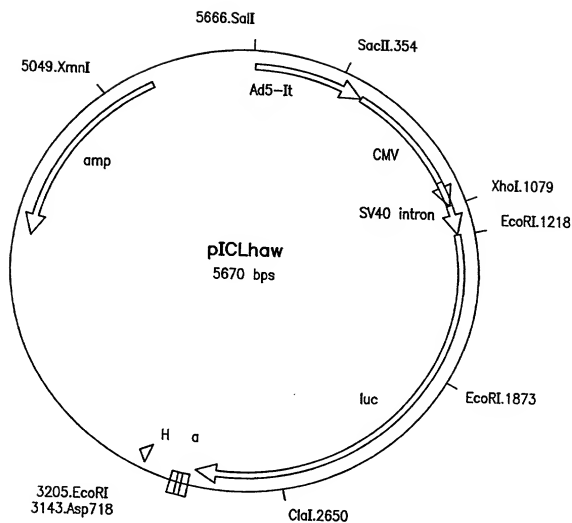


FIG. 17

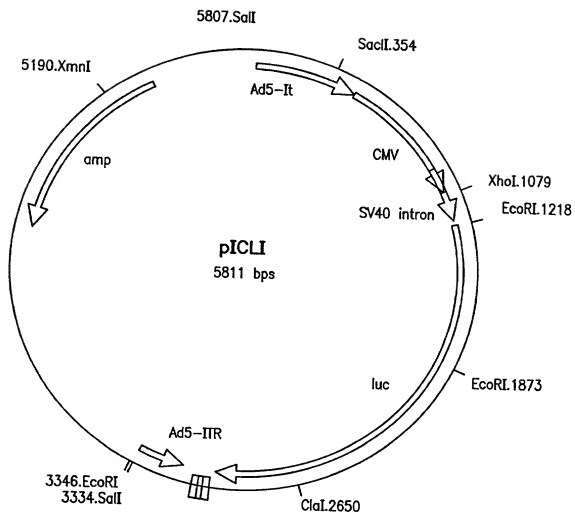


FIG. 18

10074668.021302

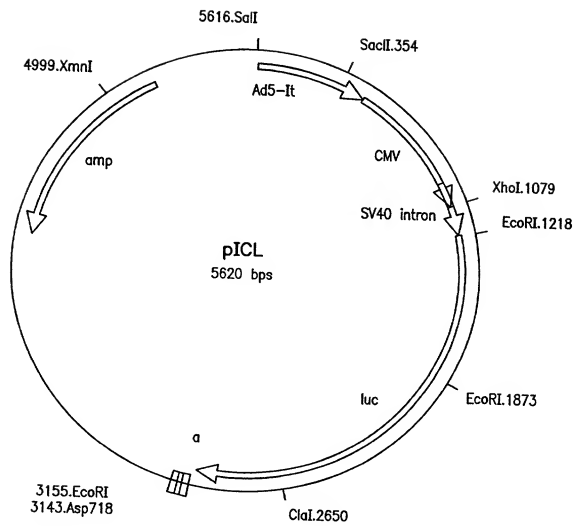


FIG. 19

# Cloned adenovirous fragments

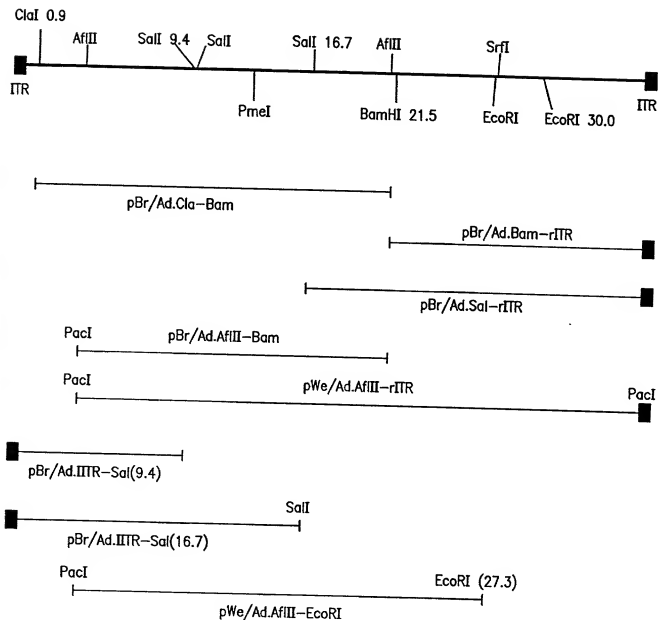


FIG. 20

Adapter plasmid pAd5/L420-HSA

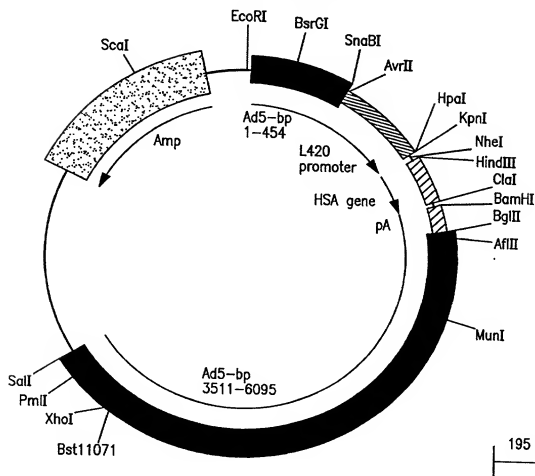


FIG. 21

Adapter plasmid pAd5/CLIP

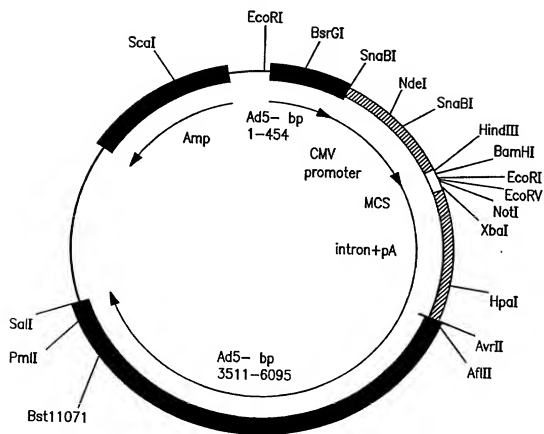


FIG. 22



# Generation of recombinant adenoviruses

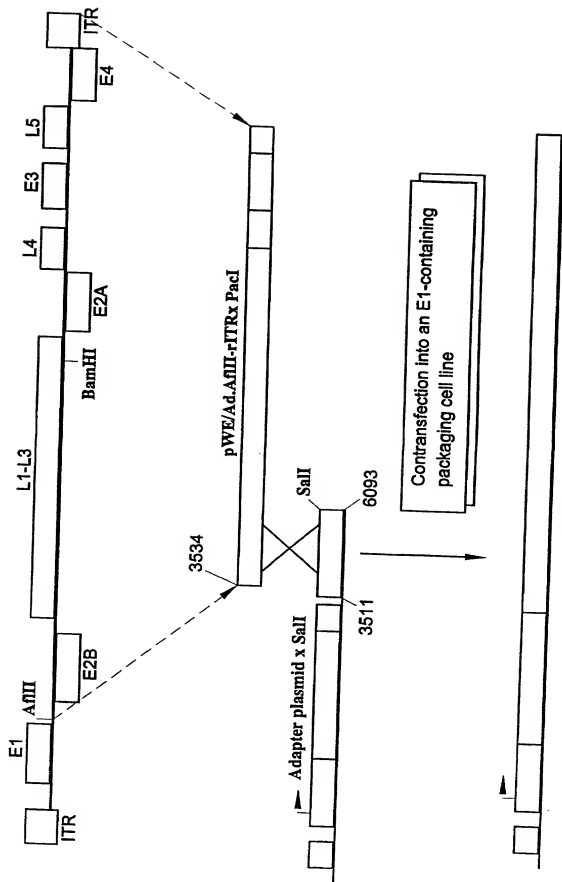


FIG. 23

# Minimal adenovirus vector pMV/L420H

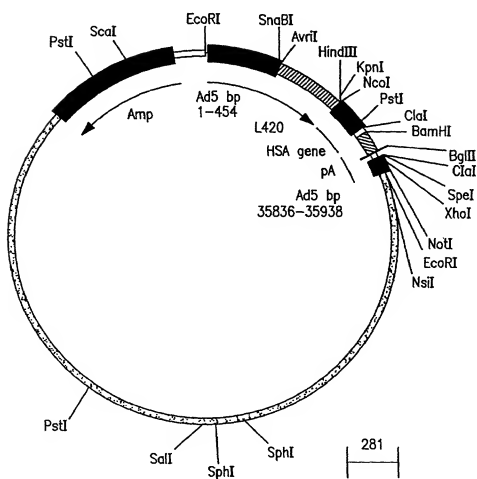


FIG. 24

# Construction of pWE/AdΔ5'

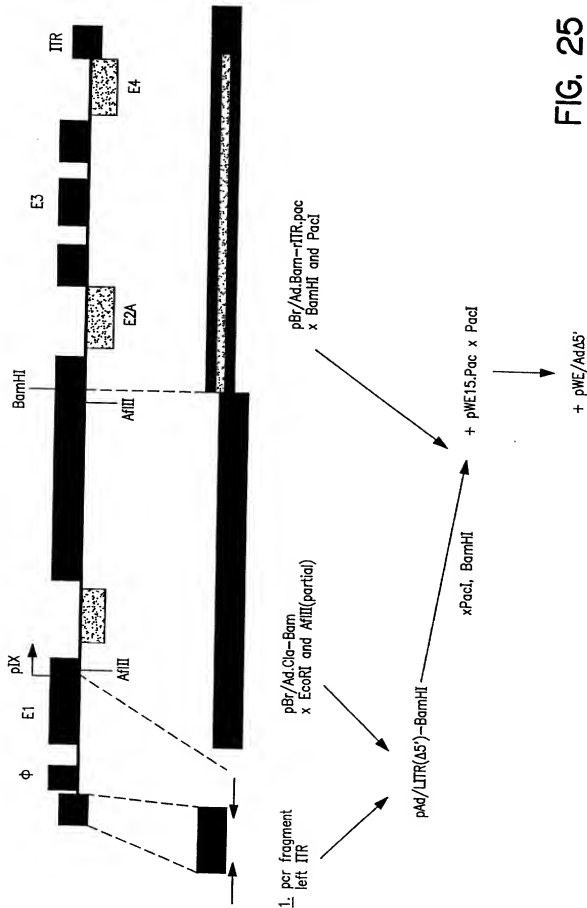


FIG. 25

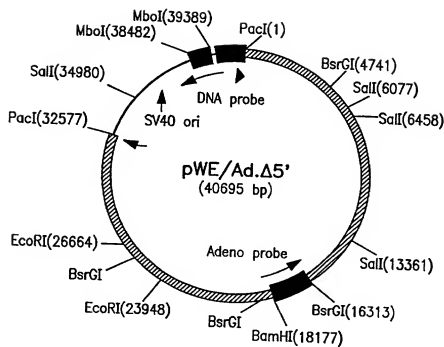


FIG. 26A

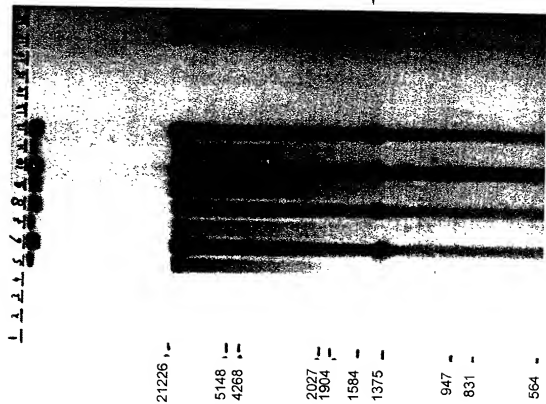


FIG. 26B

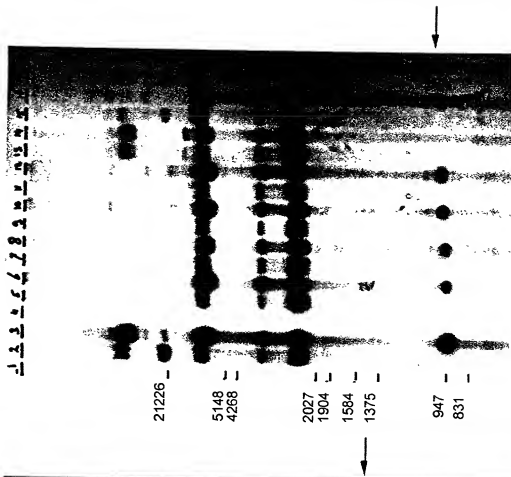
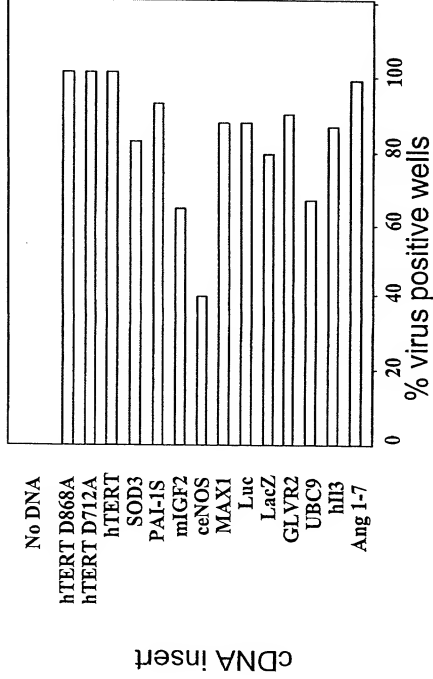


FIG. 26C



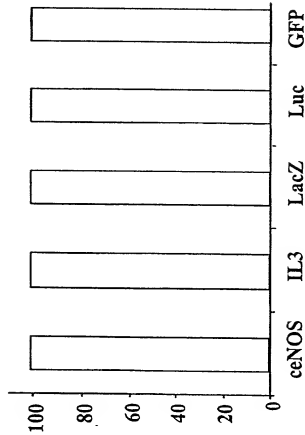
Average percentage CPE efficiency: 86 %

FIG. 27

Gene	Insert kb	Average titer $0.8 \pm 0.7 \times 10^9$ pfu/ml
• ceNOS	3.6	
• hTERT	3.5	
• hTERT D712A	3.5	
• lacZ	3.2	
• hCAT1	2.2	
• GLVR2	2.0	
• Luc	1.7	
• SOD3	1.4	
• MAX1	.550	
• hVEGF121	.511	
• hIL3	.434	
• UBC9	.412	
• ANG1-7	.104	

FIG. 28

% wells producing functional virus

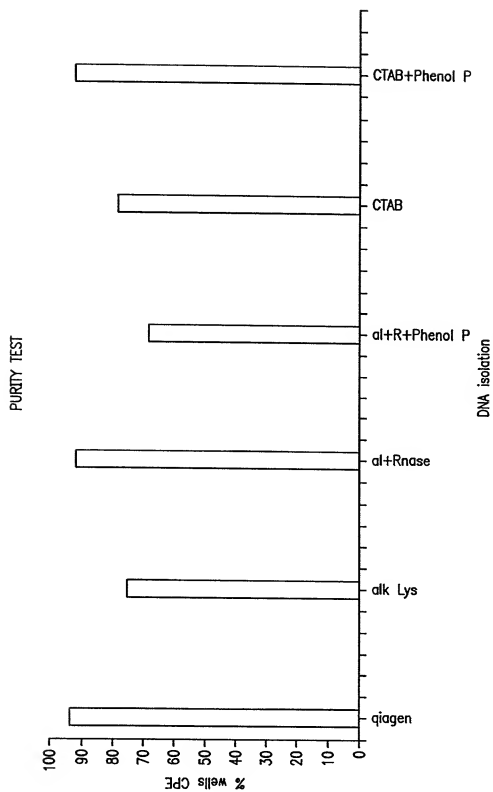


Gene	Number of CPE+ wells
ceNOS	19/19
IL3	7/7
lacZ	36/36
Luc	40/40
GFP	48/48

Gene	Number of plaques
ceNOS	9/9
IL3	9/9
lacZ	40/40
Luc	9/9
EGFP	IP
GLVR2	9/9

FIG. 29



**FIG. 30**

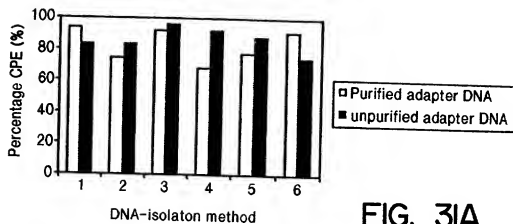


FIG. 3IA

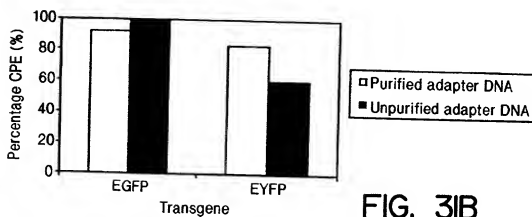


FIG. 3IB

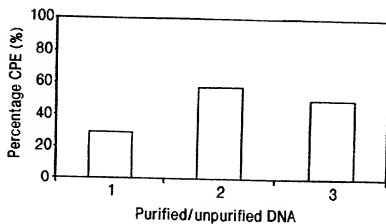


FIG. 3IC

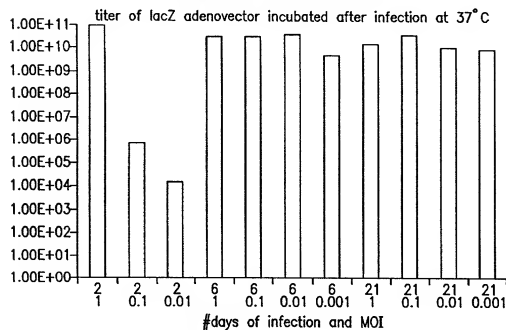
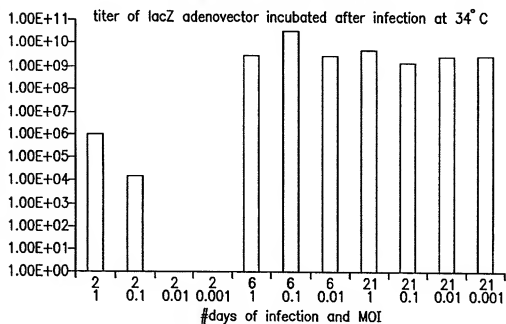
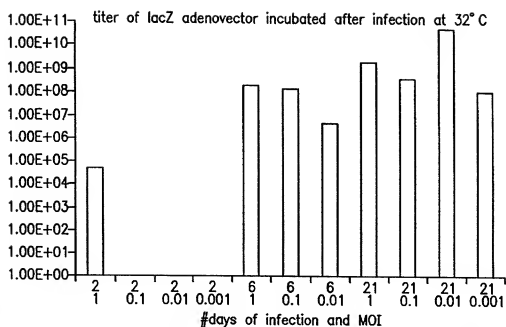


FIG. 32

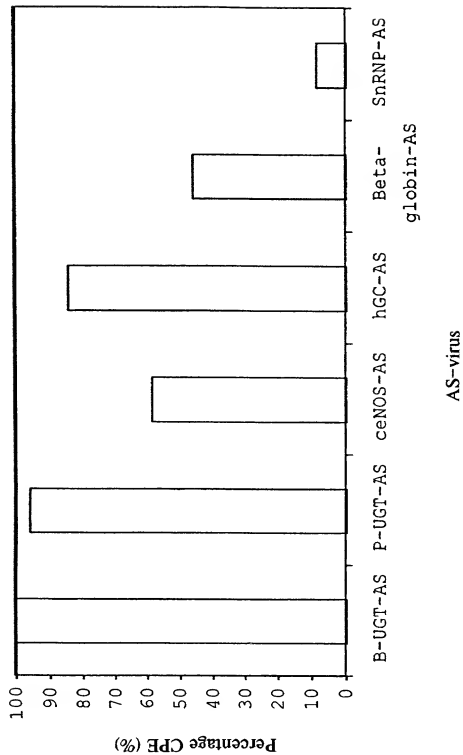


FIG. 33

2025120" 89942001

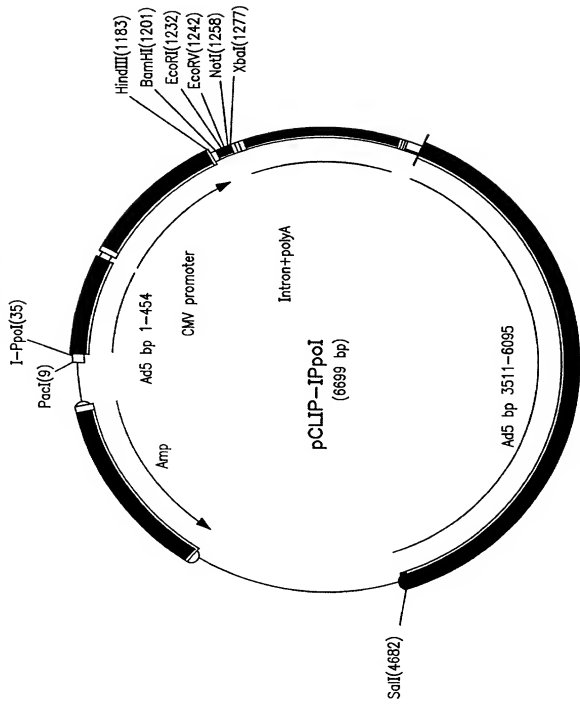


FIG. 34A

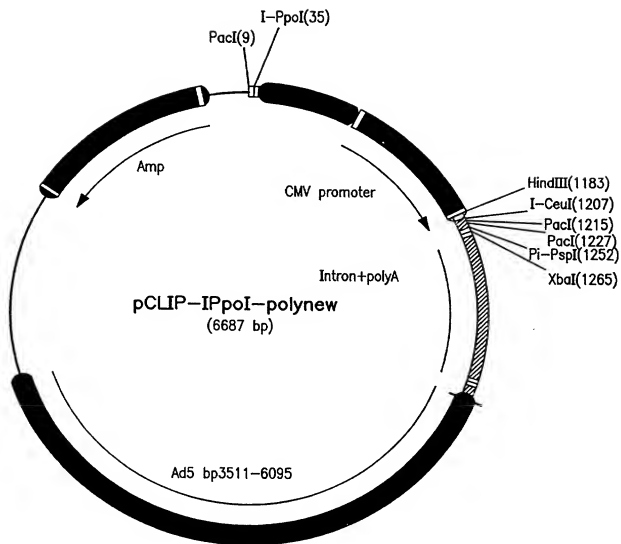


FIG. 34B

10074568.021302

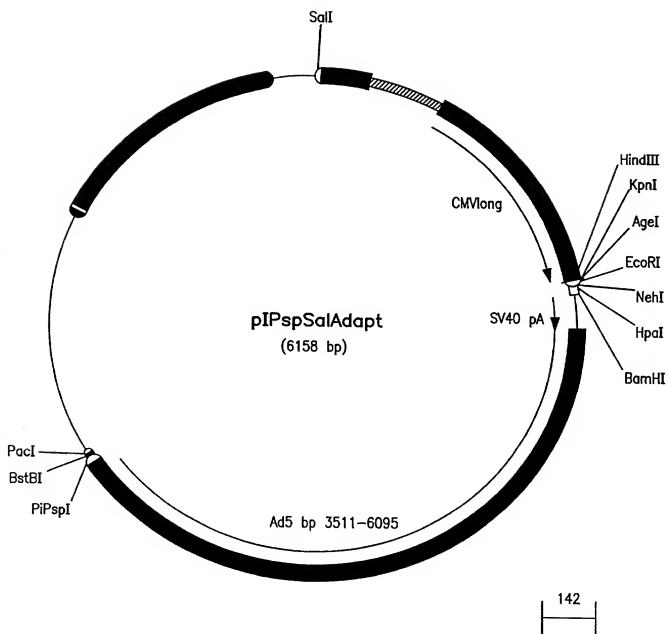


FIG. 34C

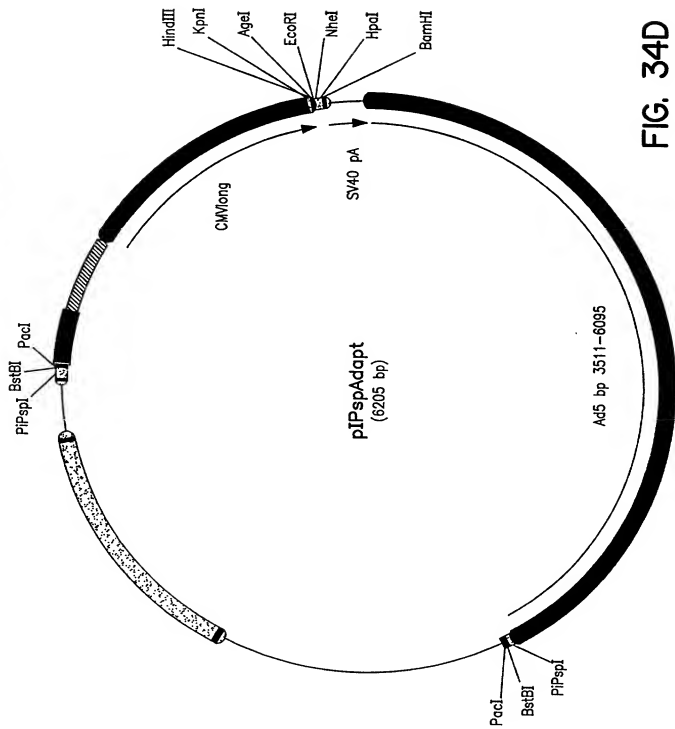


FIG. 34D



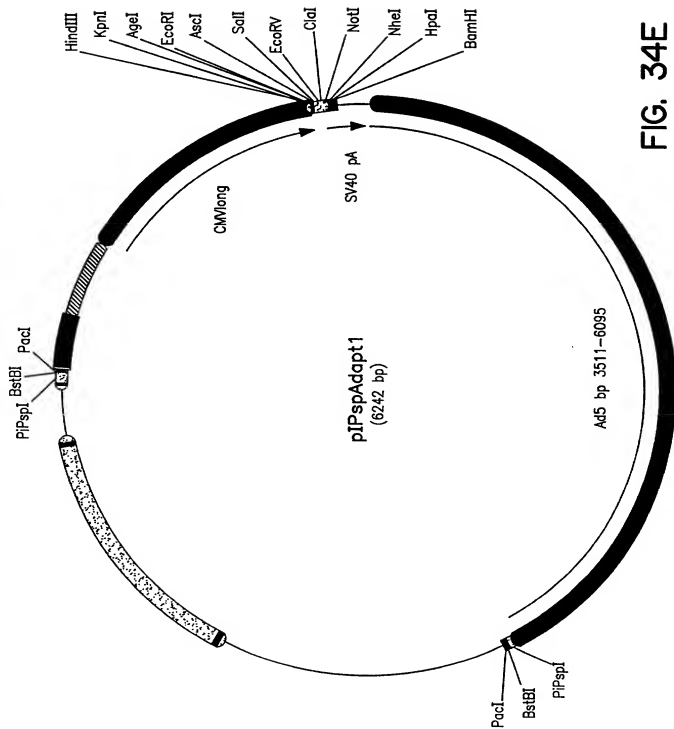


FIG. 34E

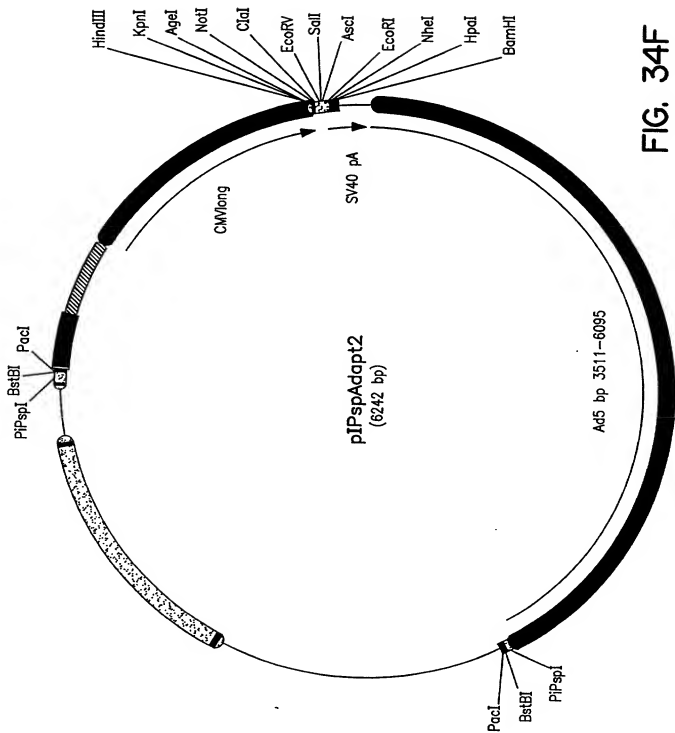


FIG. 34F

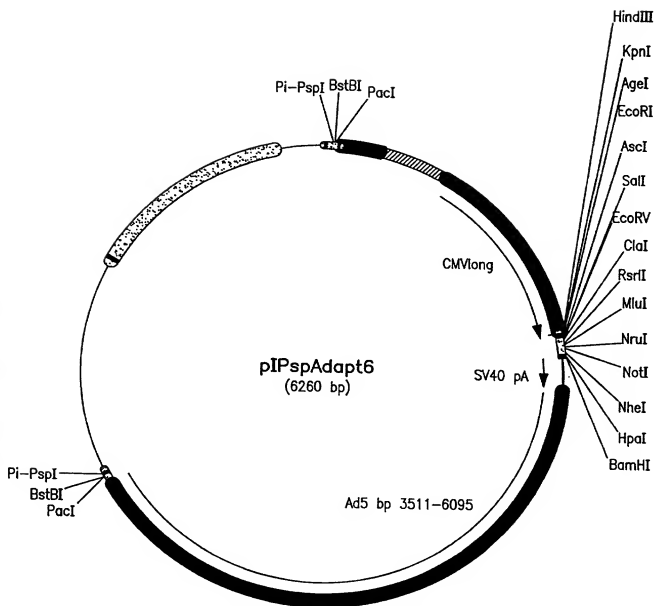


FIG. 34G

202720'6994200T

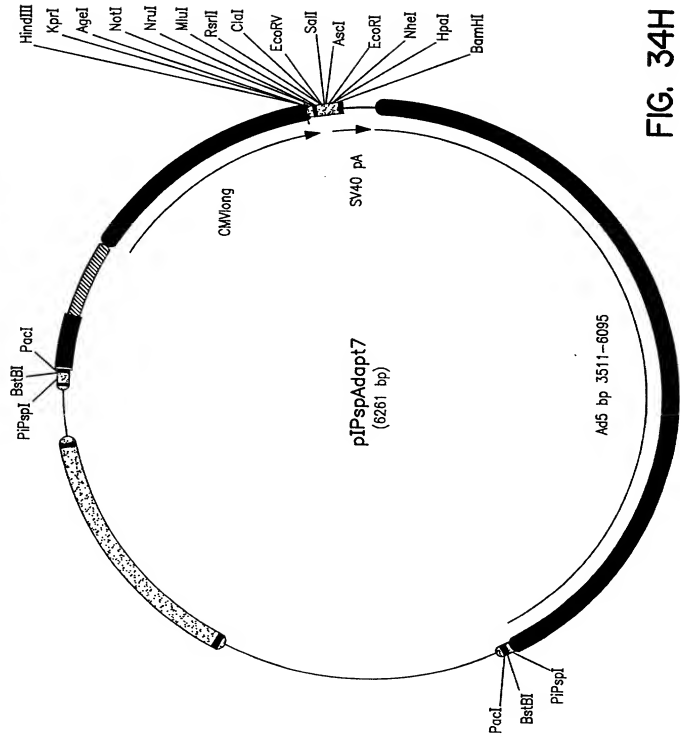


FIG. 34H

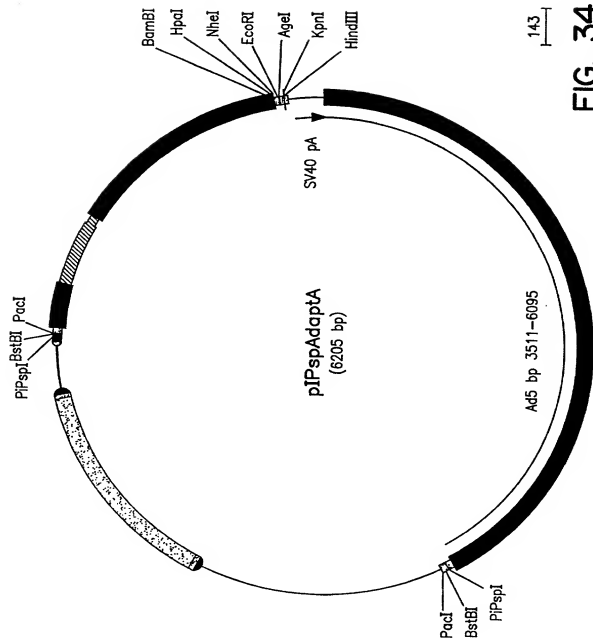


FIG. 34 I

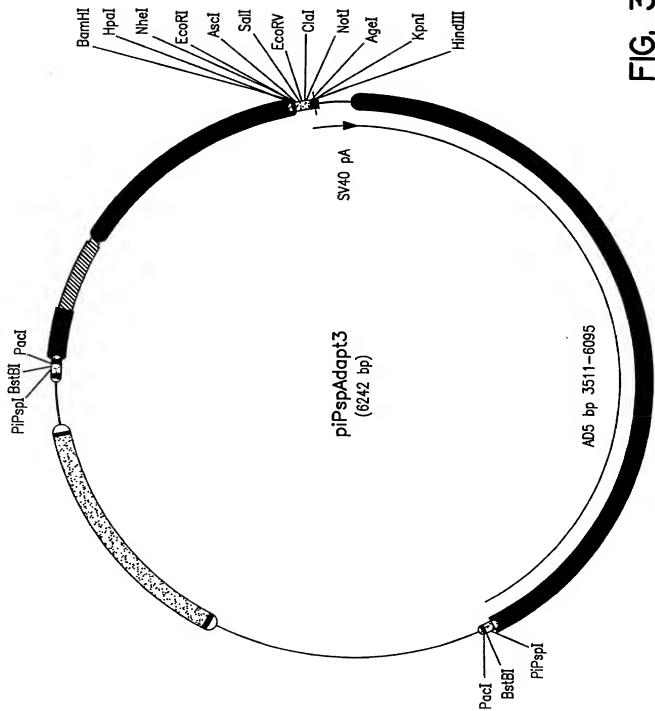


FIG. 34J

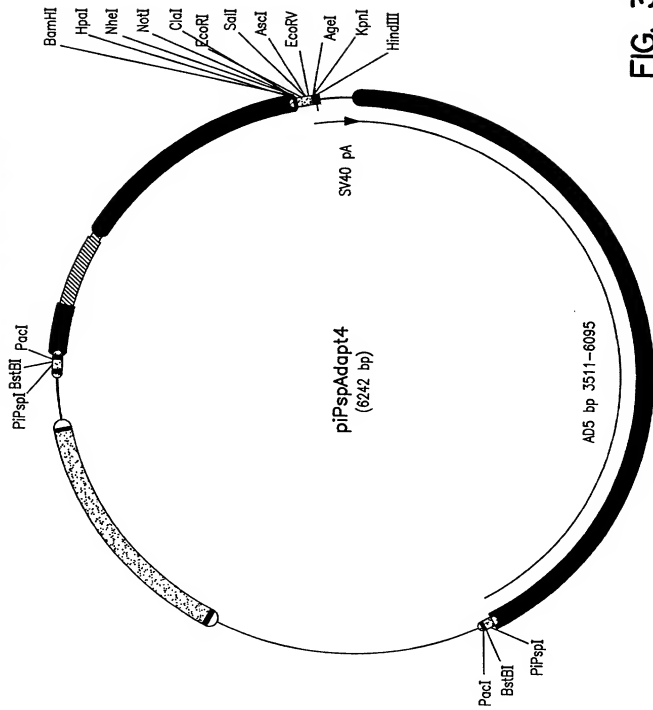


FIG. 34K

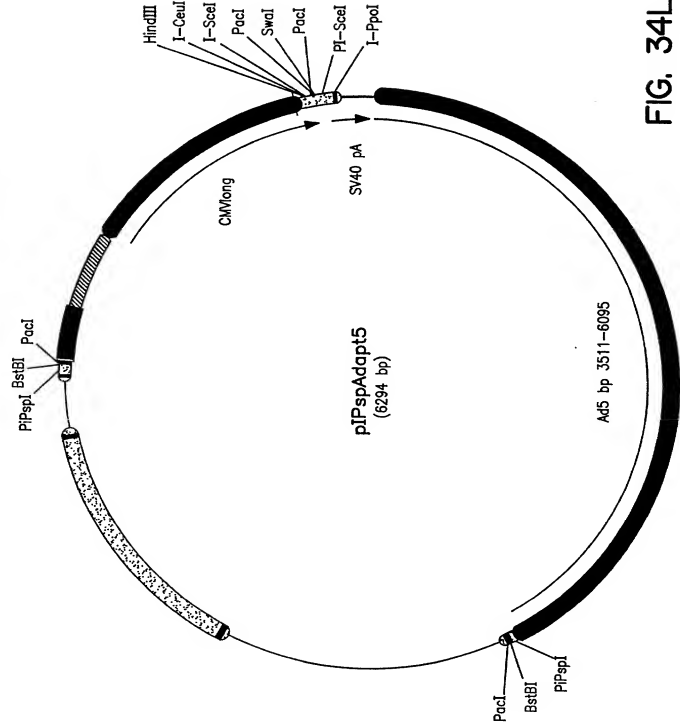


FIG. 34L



20251209 09:54:00

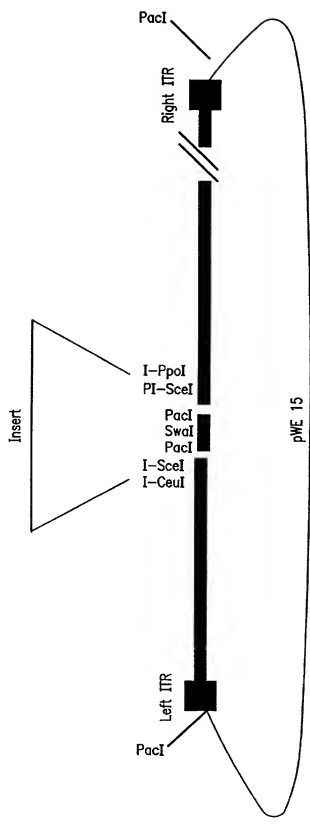


FIG. 34M

Relative amounts of wells with CPE after transfection of PER.C6/E2A cells with pCLIP-LacZ and the adapter plasmid pIPspAdapt2.

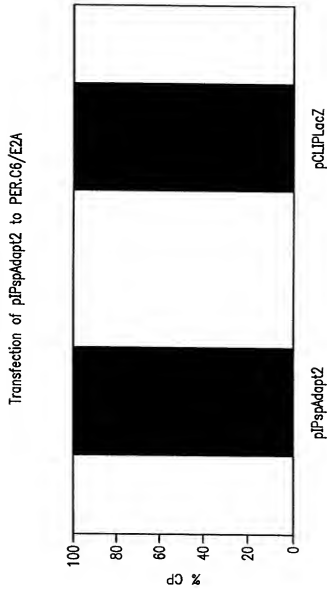


FIG. 34N

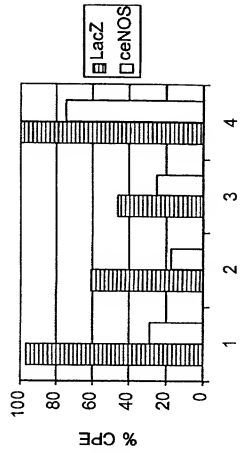


FIG. 35

# Construction total Adeno cDNA Library (1)

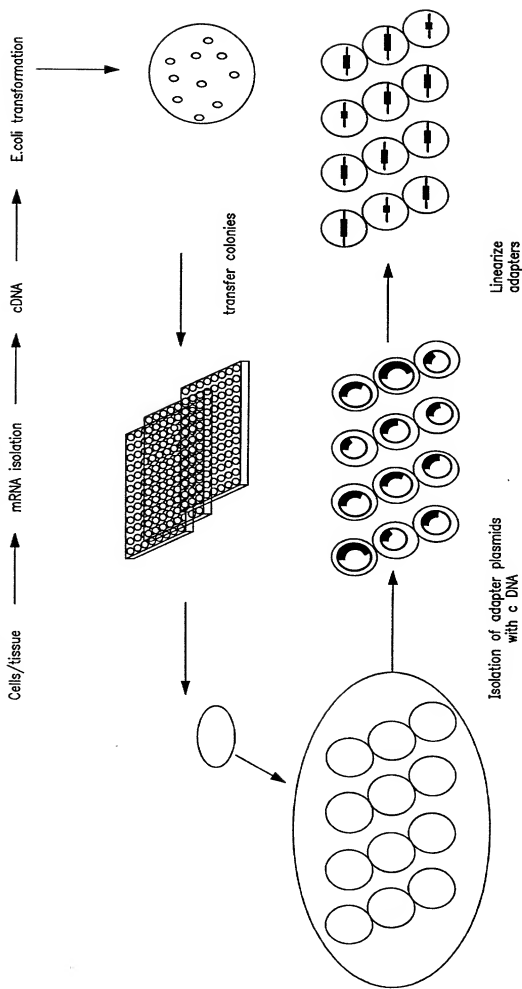


FIG. 36A

# Construction total Adeno cDNA Library (II)

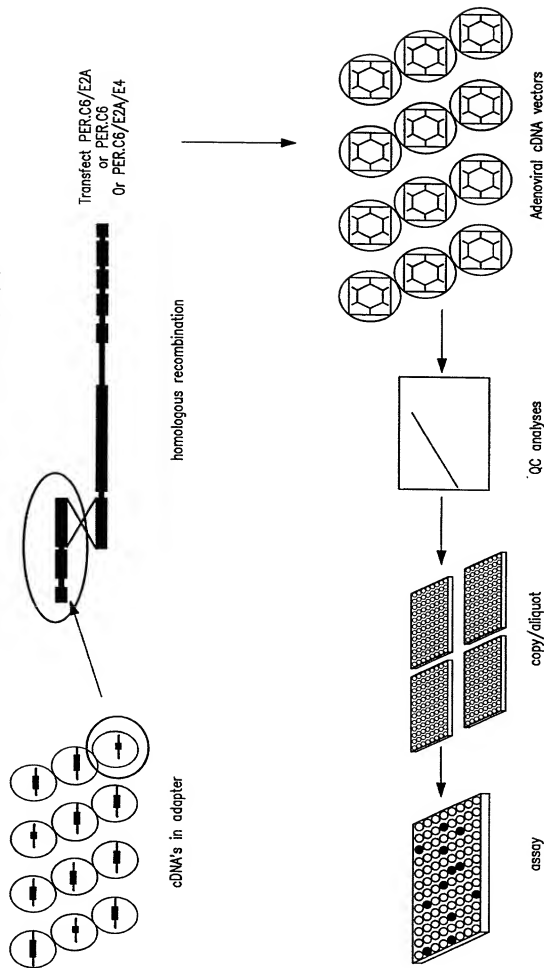


FIG. 36B

## EXAMPLE 21 384 WELL PLATE IN PROGRESS

Co-transfections on 384 well plates

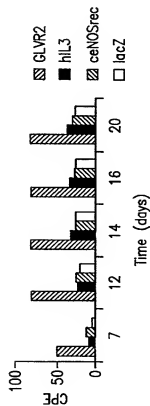


FIG. 37A

Co-transfections on 96 well plates

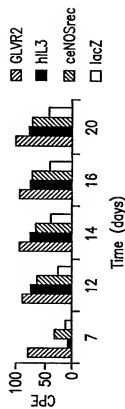


FIG. 37B

Co-transfections on 384 well plates

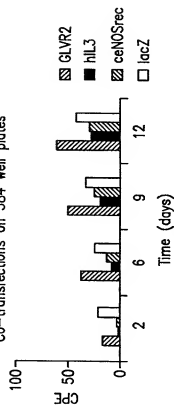


FIG. 37C

Co-transfections on 96 well plates

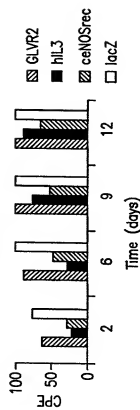


FIG. 37D

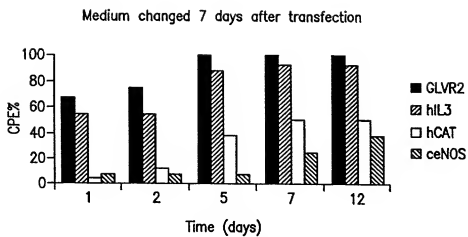


FIG. 38A

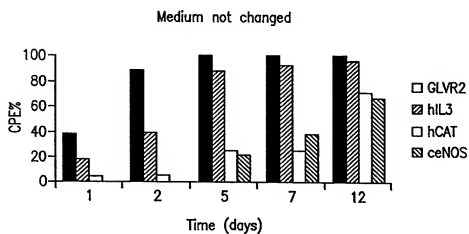


FIG. 38B

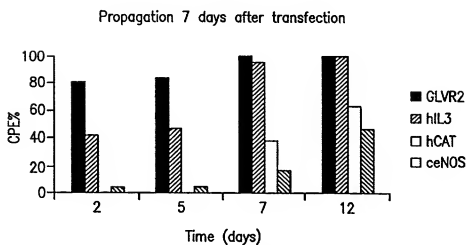


FIG. 38C

Cell titration experiment #1

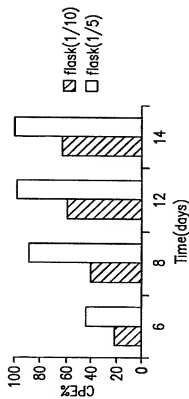


FIG. 39A

Cell titration experiment #2

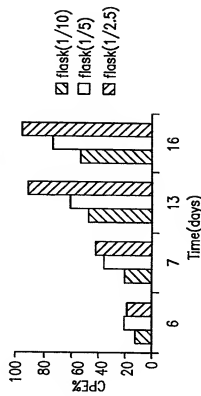


FIG. 39B

Cell titration experiment #3

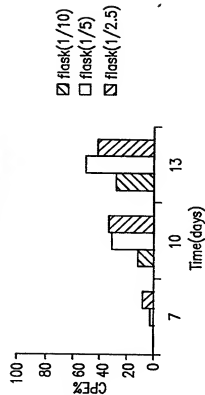


FIG. 39C



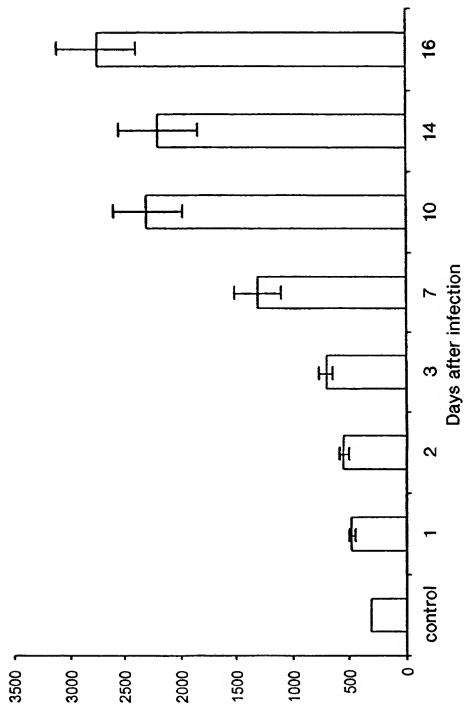


FIG. 40

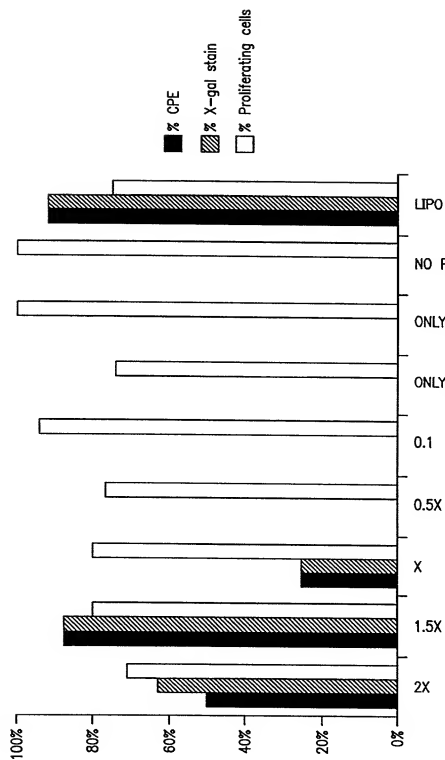


FIG. 4I

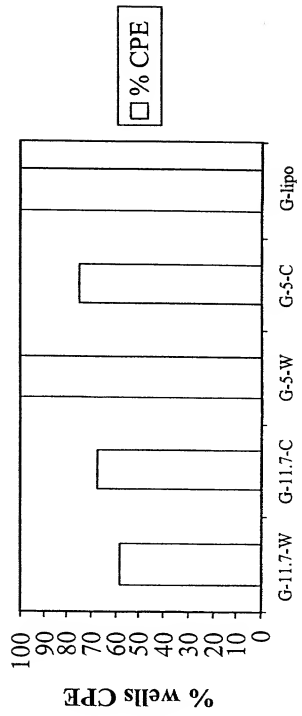
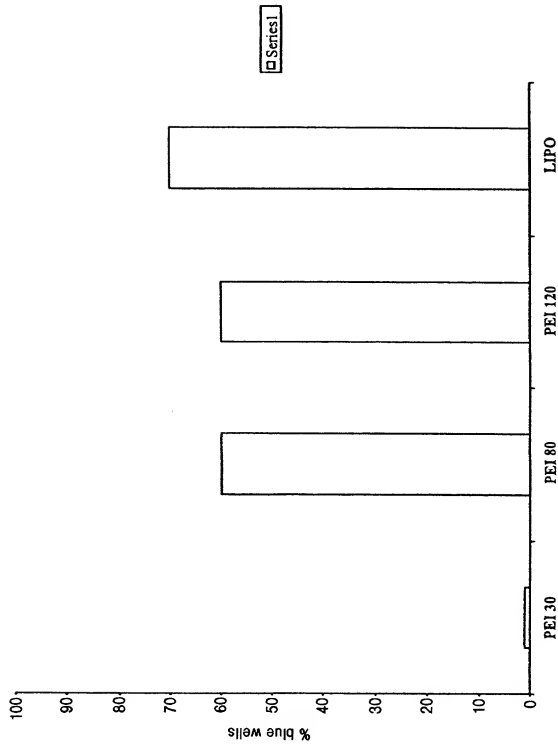


FIG. 42



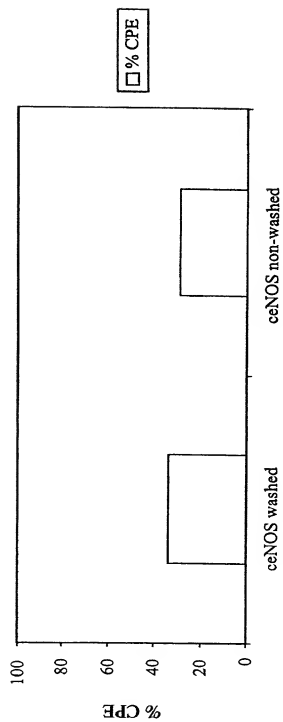


FIG. 44

Figure 45

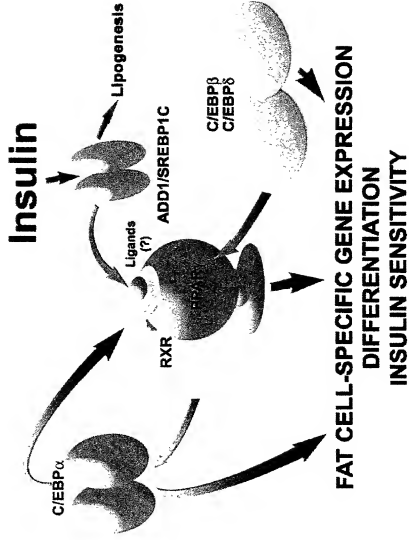


Figure 46

Downloaded from jci.org on 03/10/25. See jci.org on 03/10/25.

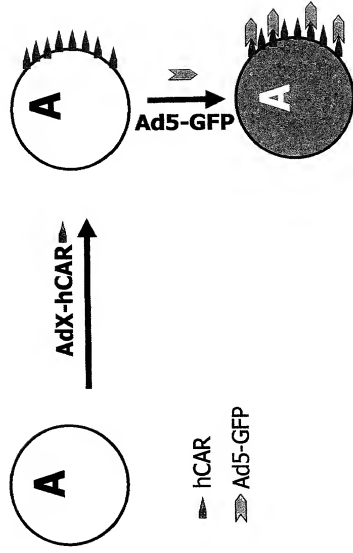


Figure 47

# Infection of human primary pre-adipocytes using Ad5C01 and Ad5C20 fiber-modified viruses

Ad5C01-eGFP



MOI:

50,000



MOI:

10,000



MOI:

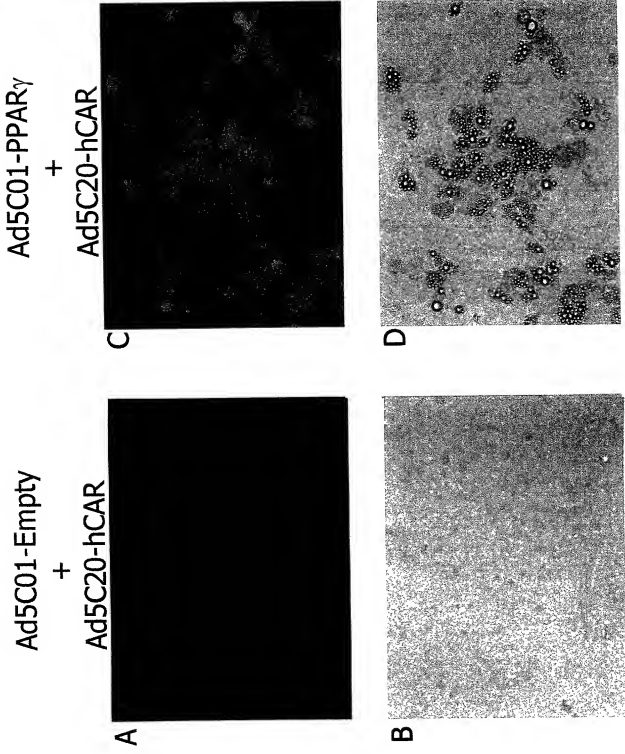
2,000

Ad5C20-eGFP





Figure 48



## Adipocyte differentiation

## Primary human mesenchymal stem cells

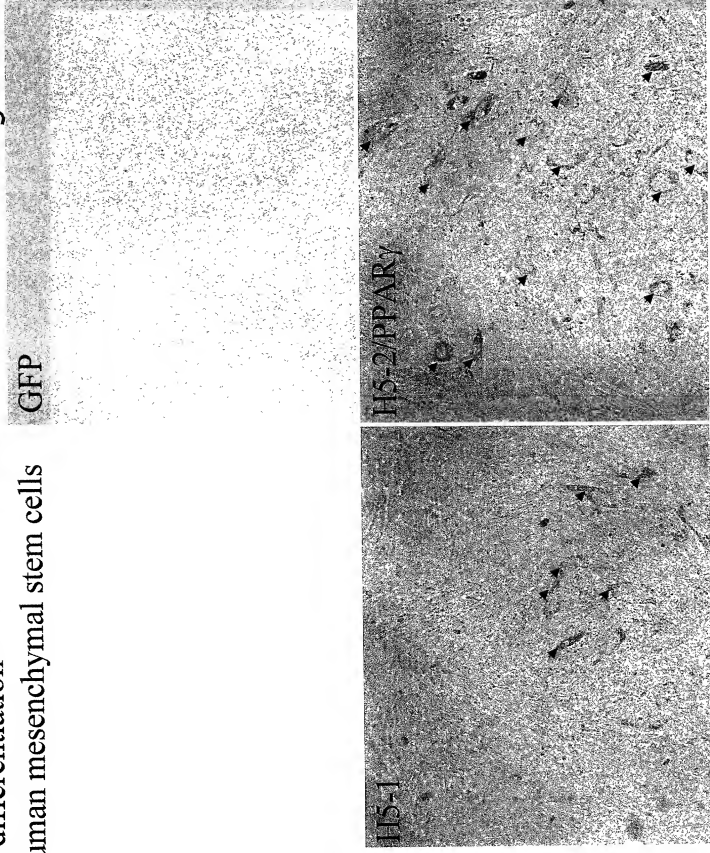


Figure 50

Adipocyte differentiation  
Mouse mesenchymal stem cell line  
C3H10T1/2

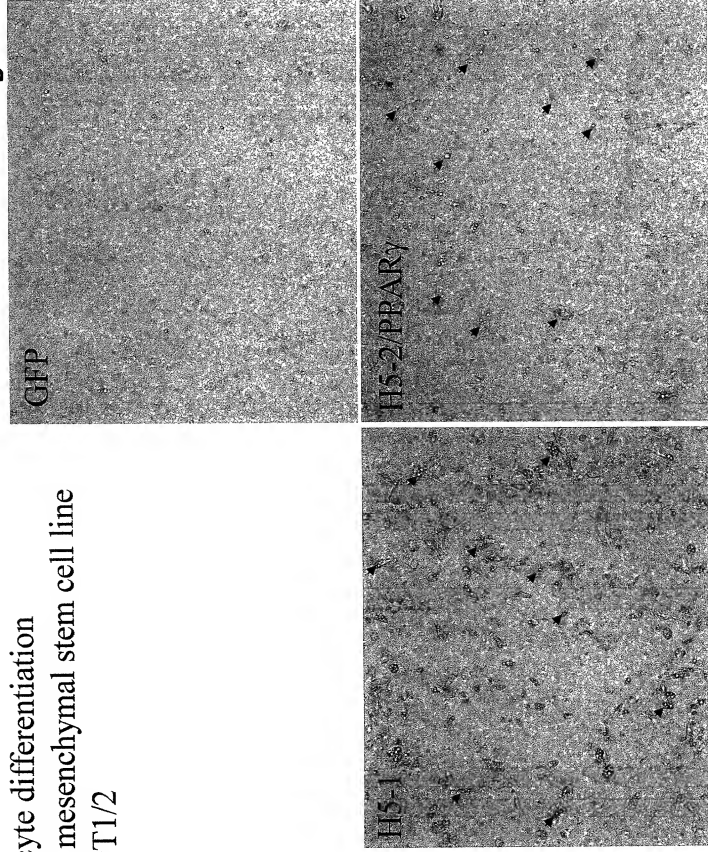
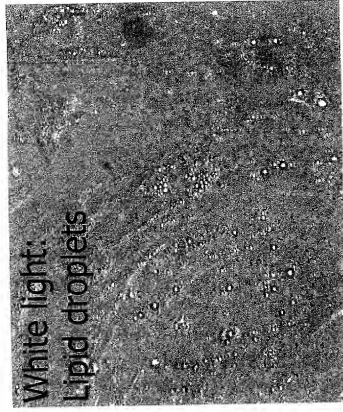


Figure 51



H5-24: adenovirally mediated expression  
of CIDEB does not induce any cell death

# FIGURE 52

H5-I DNA sequence (SEQ ID NO:14)

```

1  GCCACGCGT CCGGTTTTCT ACTTTGCCAC AGATTATCTT GTACAGCCTT TTATGGACCA
61  ATTAGCATTC CATCAATTTT ATATCTAGCA TATTTCGGGT TAGAATCCCA TGGATGTTTC
121 TTCTTTGACT ATAACAAAAT CTGGGGAGGA CAAAGGTGAT TTTCCTGTGT CCACATCTAA
181 CAAAGTCAAG ATTCCCGGCT GGACTTTTGC AGCTTCCTTC CAAGTCTTCC TGACCACCTT
241 GCACTATTGG ACTTTGGAAG GAGGTGCCTA TAGAAAACGA TTTTGAACAT ACTTCATCGC
301 AGTGGACTGT GTCCTTCGGT GCAGAAACTA CCAGATTGTA GGGACGAGGT CAAGGAGATA
361 TGATAGGCCC GGAAGTTGCT GTGCCCCATC AGCAGCTTGA CGCGTGGTCA CAGGACGATT
421 TCACTGACAC TCGGAACCTCT CAGGACTACC GTTACCAAGA GGTTAGGTGA AGTGTTTTAA
481 ACCAACGGA ACTCTTCATC TTAAACTACA CGTTGAAAT CAACCAATA ATTCGTATTT
541 AACTGAATTC TGAACCTTTC AGGAGGTACT GTGAGGAAGA GCAGGCACCA GCAGCAGAAT
601 GGGGAATGGA GAGGTGGGCA GGGGTTCCAG CTTCCCTTG ATTTTTGTCT GCAGACTCAT
661 CCTTTTTAAA TGAGACTTGT TTTCCCTCT CTTTGAGTCA AGTCAAAAT GTAGATTGCC
721 TTTGGCAATT CTTCTTCTCA AGCACTGACA CTCATTACCG TCTGTGATTG CCATTCTTTC
781 CCAAGGCCAG TCTGAACCTG AGGTTGCTTT ATCCTAAAAG TTTTAACCTC AGGTTCCAAA
841 TTCAGTAAAT TTTGGAACA GTACAGCTAT TTCTCATCAA TTCTCTATCA GTTTGAAGTC
901 AAATTTGGAT TTTCCACCAA ATTCTGAATT TGTAGACATA CTTGTACGCT CACTTGCCCC
961 AGATGCCTCC TCTGTCCTCA TTCTTCTCTC CCACACAAGC AGTCTTTTTC TACAGCCAGT
1021 AAGGCAGCTC TGTCGTGGTA GCAGATGGTC CCATTATTCT AGGGTCTTAC TCTTTGTATG
1081 ATGAAAAGAA GTGTTATGA ATCGGTGCTG TCAGCCCTGC TGCAGACCT TCTTCCACAG
1141 CAAATGAGAT GTATGCCCAA AGACGGTAGA ATTAAGAAG AGTAAATGG CTGTTGAAGC
1201 AAAAAAAAAA AAAAA

```

1007455.021302

# FIGURE 53

H5-24 DNA sequence (SEQ ID NO:16)

1 GTCGACCCAC GCCTCCGCGC CTGCAGAAAG TTGACTGCGT GGTAGGGGGC CCAGAGCAAG  
61 CCGAAGGCAA GCACGATGGC GCTCACCAGC CGGCCCAACC GCGCCCCGTG CCGCCCCGAG  
121 CCCAGCGGGG GCGCCGCGAG CCGTGCCAGC GTACGCTGT AGCAGCCGAG CATCAGCCCG  
181 AAAGGAAGCA CGAAAGCGGT GCGGTAGAC GCGCGCGGG ACAGCGAGCA ACAGGCGCGC  
241 CAGCGAGACC GCCAGCAGCA GCGCGCGGG CAGGGCCGGG CTGCCAGGCC GAGGCGCCAG  
301 GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGCTGA CGACACGCT  
361 GCGGTACATG CTGAGCGCGC ACACGTAGTA CACCGCTTG CAGCCCGCCT GCGCCAGCGG  
421 CCAGGCTGCG CGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCGCTC  
481 GCGCAGCGCC AGGTGCAGCA CAAGCGTGGC CGCCAGCGGT GCGCCCCGTG CAGGCGCCCA  
541 GCGCCGCAAG CTCACACCA CGAAGCCGTT GCCAGGCAAG CCCAGCAGCG CCGCCAGCAG  
601 CAGGAAGGCT GTGCTGTGG CCGCGGAAGT CTTCCAGCTC AGCAGTGTCT CGTTCCTTGG  
661 GGGACGGTAG CAGACCGACA TCCTTCTGGG CCTACAGGAC ACAGAAAAAA AGTGGGGAAG  
721 CTGGGGGACC CCTACAAGGA TCCTTGGCAG GAAAGCAGGG ATTGTGTCTA TTTGAGGGTT  
781 TCAGTGTCTG TGAGAGTCTC AGCTTCCATG CAACTGTCCA TCACGGCTGC AACTGAAATC  
841 AGAGCTGGGA CACAGCGCAC CAGAAGCTAA AGTCTTGATG CCATCAAGG ACATCCCTGC  
901 CCCATTACCA TCTCTGTAC GTCCACTAAT CGGCAAAAG AGAAAAGTGA GAGAAGATGA  
961 CCTAAGTGTG ACTGCAGCAG GCAGCTCTGG AAAATGAAGC CAGAGCAGTG AGCCAGCCCC  
1021 TCCTCCGACC AAGGAGGAAG GAAAGAGCAG CCCAGCACA GGAGAGAACC ACCCAGCCCCA  
1081 GAAGTTCGAG GGAAGGAACT CTCGGGTCCA CCATGGAGTA CCTCTCAGT CTGAACCCCCA  
1141 GTGACTTACT CAGGTCACTA TCTAATATAA GCTCGGAGT TTGACGGAGG GTCTGGACCT  
1201 CAGCTCCACC ACCCCAGCGA CTTTCCGTG TCTGTGATCA CAAGCGGACC ATCCGGAAAG  
1261 GCTGTACAGC TGCCACCCGC CAGGAGCTGC TAGCCAAAGC ATTGGAGACC CTACTGCTGA  
1321 ATGGAGTGCT AACCTGGTG CTAGAGGAGG ATGGAATCGC AGTGAGACAGT GAGGACTTCT  
1381 TCCAGCTGCT GGAGGATGAC ACGTGCTGA TGGTGTGCA GTCTGGTCAG AGCTGGAGCC  
1441 CTACAAGGAG TGGAGTGTGT TCATATGGCC TGGACGGGA GAGGCCCAAG CACAGCAAGG  
1501 ACATCGCCCG ATTACCTTT GACGTGTACA AGCAAAACCC TCGAGACCTC TTTGGCAGCG  
1561 TGAATGTCAA AGCCACATTC TACGGGCTCT ACTCTATGAG TTGTGACTTT CAGGACTTTG  
1621 GCCCAAGGAA AGTACTCAGG GAGCTCCTTC GTTGGACCTC CACACTGCTG CAAGGCGCTG  
1681 GCCATATGTT GCTGGGAATT TCCTCCACCC TTGTCATCG AGTGAGGGGG GCTGAGCAGT  
1741 GGCAGCAGAA GGGCGCGCTC CATTCTACT AAGGGGCTCT GAGCTTCTCG CCCGAGAATC  
1801 ATTCCAACCG ACCCACTGCA AAGACTATGA CAGCATCAAA TTTCAGGACC TGCAGACAGT  
1861 ACAGGCTAGA TAACCCACCC AATTTCCTCA CTGTCTCTG ATCCCTCTGT GACAGAACCT  
1921 TTCAGCATAA CGCCTCACAT CCCAAGTCTA TACCCTTACC TGAAGAATGC TGTCTTTTCC  
1981 TAGCCACCTT TCTAGCCTCC CACTTGCCCT GAAAGGCCAA GATCAAGATG TCCCCAGGCG  
2041 ATCTTGATCC CAGCCTGACT GCTGCTACAT CTAATCCCCC ACCAATGCCT CCGTGCCTTA  
2101 AACTCCCCAG CATACTGATG ACAGCCCTCT CTGACTTTAC CTTGAGATCT GTCTTCATAC  
2161 CTTTCCCTCT AAATAACAA AAACATTTC AATAAAAAATA TCAATATTT AAAAAAATA  
2221 AAAAAAAGGG CGGCCGCG

10074660.021302

## FIGURE 54

H5-24 ORF4 Amino Acid sequence (SEQ ID NO:15)

1 MEYLSALNPS DLLRSVSNIS SEFGRRVWTS APPQPQPFV CDHKRTIRKG  
51 LTAATRQELL AKALETLLLN GVLTLVLEED GTAVDSEDF QLEDDTCLM  
101 VLQSGQSWSP TRSGVLSYGL GRERPKHSD IARFTFDVYK QNPRDLFGSL  
151 NVKATFYGLY SMSCDFQGLG PKKVLRELLR WTSTLLQGLG HMLLGISSTL  
201 RHAVEGAEQW QKGRRLHSY 219

10074660.021302

## FIGURE 55

Region 1 of H5-24 Complementary to BLTR2 DNA sequence (SEQ ID NO:17)

```
      18 CGC CTGCAGAAGG TTGACTGCCT GGTAGGGGGC CCAGAGCAAG
61 CCGAAGGCCAA GCACGATGGC GCTCACCAGC CGGCCCCACC GCGCCCCGTG CCGCCCCGAG
121 CCCCAGCGGG CGCCCCGCAG CCGTGCCAGC GTCACGCTGT AGCAGCCGAG CATCAGCCCG
181 AAAGGAAGCA CGAAAGCGGT 200
```

10074668.021302



## FIGURE 56

Region 2 of H5-24 Complementary to BLTR2 DNA sequence (SEQ ID NO:18)

```
198 GGT GGCGGTAGAC GGCGGCCGGG ACGGCGAGCA ACAGGGCCGGC
241 CAGCCAGACC GCCAGCAGCA GGCGGCCGGC CAGGGCCGGG CTGCGCAGCC GAGGCGCCAG
301 GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGGTGA GCAGCACGCT
361 GGCGTACATG CTGAGCGCGC ACACGTAGTA CACCGCCTTG CAGCCCGCCT GGCCACGCGG
421 CCAGGCTTGC CGGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCGCTC
481 GGCCAGCGCC AGGTGCAGCA CAAGCGTGCC CGCCAGCGGT CGCCCCCGTG CAGGCCCGCA
541 GCCCGCCAAG CTCACACCA CGAAGCCGTT GCCAGGCAGC CCCAGCAGCG CCGCCACGAG
601 CAGGAAGGCT GTGCTGTGG CCGCGAAGT CTCCAGCTC AGCAGTGTCT CGTTCCTGG
661 GGGACGGTAG CAGACGACA TCCTTCTGGG CCTACAGG 698
```

1007466.024302

# FIGURE 57

Alignment of SEQ ID NO:17 with DNA sequence complementary to BLTR2 sequence.  
SEQ ID NO:17 is 100% identical to antisense BLTR2 DNA.

```

SEQ ID NO:17  18  cgccctgcagaaggttgactgcgtggttagggggccagagcaagccgaaggcaagcacgat  77
                |||
Antisense     2455 cgccctgcagaaggttgactgcgtggttagggggccagagcaagccgaaggcaagcacgat  2396
BLTR2

SEQ ID NO:17  78  ggcgctcaccagccggcccaccgcgccccgtgccgccggagccccagcggcgccccg  137
                |||
Antisense     2395 ggcgctcaccagccggcccaccgcgccccgtgccgccggagccccagcggcgccccg  2336
BLTR2

SEQ ID NO:17  138  cagccgtgccagcgtcacgctgtagcagccgagcatcagccgaaagggaagcacgaaagc  197
                |||
Antisense     2335 cagccgtgccagcgtcacgctgtagcagccgagcatcagccgaaagggaagcacgaaagc  2276
BLTR2

SEQ ID NO:17  198  ggt  200
                |||
Antisense     2275 ggt  2273
BLTR2

```

10074688.021302

# FIGURE 58

Alignment of SEQ ID NO:18 with DNA sequence complementary to BLTR2 sequence.

SEQ ID NO:18 is 100% identical to antisense BLTR2 DNA.

```

SEQ ID NO:18 198 ggtggcggtagacggcgcgcgggagcgagcaacaggcgccagccagaccgccagca 257
                  |||
Antisense     2195 ggtggcggtagacggcgcgcgggagcgagcaacaggcgccagccagaccgccagca 2136
BLTR2

SEQ ID NO:18 258 gcaggcgcgccggccaggcgcggtgcgagcgaggcgccagggaaggcgcggtgactg 317
                  |||
Antisense     2135 gcaggcgcgccggccaggcgcggtgcgagcgaggcgccagggaaggcgcggtgactg 2076
BLTR2

SEQ ID NO:18 318 cgaggcagcgctgcaggtgagcaggccggtgagcagcacgctggcgctacatgctgagcg 377
                  |||
Antisense     2075 cgaggcagcgctgcaggtgagcaggccggtgagcagcacgctggcgctacatgctgagcg 2016
BLTR2

SEQ ID NO:18 378 cgcacacgtagtacacgccttgacgcccgcctggcccagcgccaggcctgccgggtca 437
                  |||
Antisense     2015 cgcacacgtagtacacgccttgacgcccgcctggcccagcgccaggcctgccgggtca 1956
BLTR2

SEQ ID NO:18 438 ggaaggccacaagaagagcgcgctgagcagcagcacgcgcgcctggccagcgccagggtgca 497
                  |||
Antisense     1955 ggaaggccacaagaagagcgcgctgagcagcagcacgcgcgcctggccagcgccagggtgca 1896
BLTR2

SEQ ID NO:18 498 gcacaagcgtggccgcagcggtcgcccccgctgcaggccgcagcccgccaagctccaca 557
                  |||
Antisense     1895 gcacaagcgtggccgcagcggtcgcccccgctgcaggccgcagcccgccaagctccaca 1836
BLTR2

SEQ ID NO:18 558 ccacgaagccgttgccaggcagccccagcagcgccgcagcagcaggaaggctgtgcctg 617
                  |||
Antisense     1835 ccacgaagccgttgccaggcagccccagcagcgccgcagcagcaggaaggctgtgcctg 1776
BLTR2

SEQ ID NO:18 618 tggcccgogaagttcttcagctcagcagtgctcgttccctgggggacggtagcagaccg 677
                  |||
Antisense     1775 tggcccgogaagttcttcagctcagcagtgctcgttccctgggggacggtagcagaccg 1716
BLTR2

SEQ ID NO:18 678 acatccttctgggcctacagg 698
                  |||
Antisense     1715 acatccttctgggcctacagg 1695
BLTR2

```

10074658.021302